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Increased levels of immunological markers in the respiratory tract but not in serum correlate with active pulmonary mycobacterial infection in mice

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Abstract

Immunological tests for the diagnosis of tuberculosis (TB) have relied mostly on detection of immune markers in serum or release of cytokines by mononuclear cells *in vitro*. These tests, although useful, sometimes fail to discriminate between active infection and contact with mycobacteria or vaccination. TB is primarily a disease of the lung, and therefore identification of immunological markers in the respiratory tract will be more likely to reflect the infection status or disease activity. In this study, it is demonstrated that active infection of mice with *Mycobacterium bovis* bacille Calmette-Guérin (BCG), but not exposure to heat-killed BCG, induced production of interleukin-12 (IL-12), interferon-γ (IFN-γ) or soluble tumour necrosis factor receptors (sTNFRs) locally in the lungs, as detected in bronchoalveolar lavage (BAL) fluid. There was a strong correlation between bacterial growth in the lung and levels of sTNFRs, and to some extent IL-12 and IFN-γ, in BAL fluid. Furthermore, sTNFR levels increased significantly in BAL fluid after reactivation of controlled infection with dexamethasone, and this correlated with increased bacterial growth in the lungs. Finally, infection, but not exposure to non-replicating mycobacteria, induced specific IgG and IgA in BAL fluid. Elevated levels of all biomarkers measured were also detected in the serum, but correlation with infection was not as clear as in the case of BAL fluid. Taken together, the detection of sTNFRs and mycobacterium-specific antibodies, especially IgA, locally in the lungs could be used as immunological markers for the diagnosis of TB.

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Introduction

It is estimated that between 5% to 10% of immunocompetent individuals are susceptible to tuberculosis (TB), and of these, 85% develop pulmonary disease [1] in the first 2 years after exposure. Immunity to TB depends on several factors, including cytokines, chemokines, antibodies, macrophages, neutrophils, several T-cell subsets, and specific patterns of T-cell migration [2,3]. The infected host generates a Th1 type of immune response, in which mycobacterial antigen-specific T-lymphocytes are recruited to the lungs, and play a significant role in protection against *Mycobacterium tuberculosis* infection [4]. Interferon-γ (IFN-γ) is considered to be an important correlate of protective immunity against *M. tuberculosis* infection, and, together with other cytokines such as interleukin-12 (IL-12) and tumor necrosis factor (TNF), plays a critical role in the development of protective granulomas and induction of antimycobacterial activity in macrophages [2–5]. Even though the correlation is less clear-cut, specific antibodies, particularly IgA, in mucosal secretions have been related to protective immunity against pulmonary infections [6].

Improvement in the currently available diagnostic tools is of utmost importance for the control of the global TB epidemic. In the vast majority of low-income and middle-income countries, where TB is prevalent, diagnosis primarily relies on the microscopic identification of acid-fast bacilli in unprocessed sputum smears. Although acid-fast staining is

relatively quick, the sensitivity can be highly variable [7]. Although mycobacterial culture is the ultimate proof of active *M. tuberculosis* infection, and is often used as a reference method for diagnosis, because of its high sensitivity and specificity [8], it is slow and involves direct handling of live bacteria. The tuberculin skin test is used widely in several countries as the standard method for the diagnosis of latent TB. The interpretation of this test, which is based on the detection of a delayed-type hypersensitivity reaction to purified protein derivative after intradermal injection, can, however, be affected by factors such as age, exposure to environmental mycobacteria, or *Mycobacterium bovis* bacille Calmette–Guérin (BCG) vaccination [9].

As compared with the traditional tuberculin skin test and microscopy for acid-fast bacilli, newer IFN-γ release assays [10] and nucleic acid amplification tests [11] lead to more rapid detection of *M. tuberculosis* infection. However, the sensitivity estimates for commercial nucleic acid amplification tests in respiratory specimens are highly variable [11]. In addition, the infrastructure required to collect specimens, deliver them to the correct laboratory and perform these tests make it difficult to establish in countries with severely limited resources.

Until recently, researchers have looked mainly in serum for identification of specific mycobacterial antibodies as markers of infection [12]. With regard to the use of cytokines or cytokine receptors as surrogate markers of mycobacterial infection, levels have been assessed mainly in the serum of TB patients [13,14]. Overall, serological methods have been used with limited success. As TB is mainly a disease of the lungs, antibodies and cytokines detected locally in the lungs and associated fluids would be more likely to reflect infection status.

In this study, we have followed the production of various immunological parameters in mice, using BCG as the infectious agent. We aimed to identify markers of cellular activation and specific mycobacterial antibodies locally in bronchoalveolar lavage (BAL) fluid and in serum that, in combination or separately, might be indicative of active mycobacterial infection. We demonstrated that active infection of mice, but not exposure to non-replicating mycobacteria (heat-killed BCG (hk-BCG) or BCG lysate), resulted in increased levels of IL-12, IFN-γ and soluble TNF receptors (sTNFRs) in the BAL fluid. Moreover, infection, but not exposure to non-replicating mycobacteria, induced production of specific IgA antibodies in the respiratory tract that were undetectable in serum. Despite the obvious differences, mouse models of BCG infection have been extensively used, and we discuss here the potential translation of our findings to the diagnosis of human TB.

Materials and Methods

Mice

The studies were performed using 8–12-week-old female BALB/c mice purchased from Taconic Europe, Denmark, and housed in pathogen-free conditions. All animals were kept at the Animal Department of the Arrhenius Laboratories, Stockholm University, Sweden. Experiments were performed in accordance with the guidelines of the animal research ethics board at Stockholm University.

Bacteria

BCG (Pasteur strain), obtained from A. Williams, HPA, Salisbury, UK, was grown in Middlebrook 7H9 broth (Difco, Sparks, MD, USA) with glycerol supplemented with albumin–dextrose–catalase, at 37°C, to the mid-log phase. Aliquots were frozen in phosphate-buffered saline (PBS) with toxiglycerol at −70°C. Three vials picked randomly from the stock were thawed, and serially diluted in plating buffer (PBS with 0.05% Tween-80 (v/v)); CFUs were counted at 2–3 weeks after plating on Middlebrook 7H11 agar (Difco), with glycerol and oleic acid–albumin–dextrose–catalase enrichment.

To prepare hk-BCG, 10^7 CFU/mL of BCG were autoclaved at 121°C for 15 min, washed once, and resuspended in sterile PBS before use. For the preparation of BCG lysate, 10^7 CFU/mL bacteria were pelleted by spinning at 8000***g***, resuspended in 0.05% Tween-80 in PBS, and washed twice. The bacteria were then resuspended in 5 mL of ice-cold PBS and sonicated (Sonifier B12; Branson Sonic Power, Danbury, CT, USA) on ice for 14 cycles of 1 min each, as described by Power *et al.* [15]. The sonicated suspension was spun at 12 000***g*** for 30 min at 4°C to remove particulate matter, and the supernatant containing soluble antigens (referred to as BCG lysate) was collected and stored at −20°C. The protein concentration of the lysate was determined using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. Soluble bovine serum albumin fraction V was used as a protein standard.

Single mycobacterial antigens

The antigen 85 complex (Ag85) was obtained from Colorado State University. The recombinant antigens 38 kDa, 19 kDa, and 16 kDa, commonly used in the serodiagnosis of TB [16–18], were all obtained from LIONEX Diagnostics & Therapeutics GmbH, Braunschweig, Germany.

Experimental infection or treatment with non-replicating BCG, determination of CFUs, and reactivation of controlled infection

Mice were infected intranasally with 10^7 CFU of live BCG, or treated with 10^7 CFU of non-replicating BCG (BCG lysate or hk-BCG) in 30 μL of PBS, or PBS alone as control. Mice were anaesthetized with isofluorane (Baxter Medical AB, Kista, Sweden), and administration was carried out by inoculation of 15 μL per nostril given in two doses. Mice were allowed to breathe the suspension into the lungs naturally [19]. At various time-points, mice were killed by cervical dislocation. Lungs, spleen and liver were removed aseptically, placed in 2 mL of plating buffer and homogenized in glass homogenizers. Serial dilutions of the organ homogenates were plated on Middlebrook 7H11 agar plates with oleic acid–albumin–dextrose–catalase enrichment, and incubated at 37°C. The number of CFUs was determined 2–3 weeks after plating. Reactivation of controlled infection was performed as described by Lowrie *et al.* [20], with some modifications. Mice were injected intraperitoneally at week 10 after infection (day 0) with 6 mg of dexamethasone (DXM) (Sigma, St Louis, MO, USA) per kg body weight, and the treatment was repeated at days 2, 4, and 6, giving a total of four doses.

Sample collection

Serum and BAL fluid were collected from each group of mice at different time-points. To obtain sera, mice were bled from the tail vein, and serum was collected after centrifugation. BAL fluid was obtained by flushing 1.5 mL of PBS into the lungs of the killed mice. All samples were kept at −20°C until use.

Cytokine and cytokine receptor detection assays

TNF and sTNFR ELISAs were performed using the commercially available DuoSet ELISA Development Systems (R&D Systems Europe, Abingdon, UK), according to the manufacturer's recommendations, with slight modifications. Streptavidin conjugated to alkaline phosphatase (MABTECH, Nacka, Sweden) was used instead of horseradish peroxidase, at a 1 : 1000 dilution. IL-12 (p70) and IFN-γ ELISA was performed using commercially available kits (MABTECH). The enzyme–substrate reaction was developed using *p*-nitrophenyl phosphate (Sigma). Optical density was read in a multiscan plate reader (Anthos Labtech Instruments, Salzburg, Austria) at 405 nm. On the basis of previous results, sample dilutions of BAL fluid started at 1 : 2, 1 : 5, 1 : 2, 1 : 5 and 1 : 50 for IFN-γ, IL-12, TNF, sTNFR1 and sTNFR2, respectively. Serum dilutions started at 1 : 5, 1 : 10, 1 : 5, 1 : 40 and 1 : 100 for IFN-γ, IL-12, TNF, sTNFR1 and sTNFR2, respectively.

Detection of antibodies in serum and BAL fluid

Antibodies in serum and BAL fluid were analysed by ELISA as described previously [6]. ELISA plates (high binding; Costar, Corning, NY, USA) were coated with either BCG lysate (20 μg/mL) or Ag85, 38 kDa, 19 kDa or 16 kDa (2 μg/mL) in carbonate–bicarbonate buffer (pH 9.6) overnight at room temperature (RT). After washing, diluted samples were incubated overnight at RT. Plates were then washed and incubated for 2 h at RT with alkaline phosphatase-labelled goat anti-mouse IgG or IgA (Southern Biotech, Birmingham, AL, USA), and the enzyme–substrate reaction was developed using *p*-nitrophenyl phosphate as substrate. Optical density was measured in a multiscan reader at 405 nm. Non-specific antibodies were determined in parallel, using bovine serum albumin fraction V as an unrelated antigen, and these values were deducted from those obtained for specific anti-BCG antibodies. For the detection of antibodies in BAL fluid or serum, single sample dilutions were chosen on the basis of initial results, starting from 1 : 5 (BAL fluid) and 1 : 100 (serum).

Statistical analysis

One-way analysis of variance (ANOVA) with Bonferroni *post hoc* tests was used to compare bacterial growth in the lungs or cytokine and soluble receptor levels in BAL fluid, taking day 3 as the reference point. Spearman's rank correlation was used to establish the correlation between bacterial burden and cytokine or soluble cytokine receptor levels. Data are presented as the mean value ± standard deviation, and are considered to be significant when $^*p < 0.05$. All analyses were performed with SigmaStats software Systat Software Inc., San Jose, CA, USA.

Results

Intranasal infection, but not treatment of mice with non-replicating BCG, induces elevated levels of IL-12, IFN-γ and sTNFRs in the lungs

TNF, IL-12 and IFN-γ are essential components of the protective immune response against mycobacterial infection, and elevated levels of these cytokines in the lungs, the principal organ involved during *M. tuberculosis* infection, could be indicative of disease activity. To assess this, BAL fluid from mice infected intranasally with BCG or treated with non-replicating BCG was collected at different time-points and analysed for IL-12, IFN-γ and TNF. Bacterial load was quantified in lung, spleen and liver homogenates (Fig. 1a).

Live, but not non-replicating, BCG induced production of IL-12 and IFN-γ in the lungs (Fig. 1b–c). The amount of IL-12

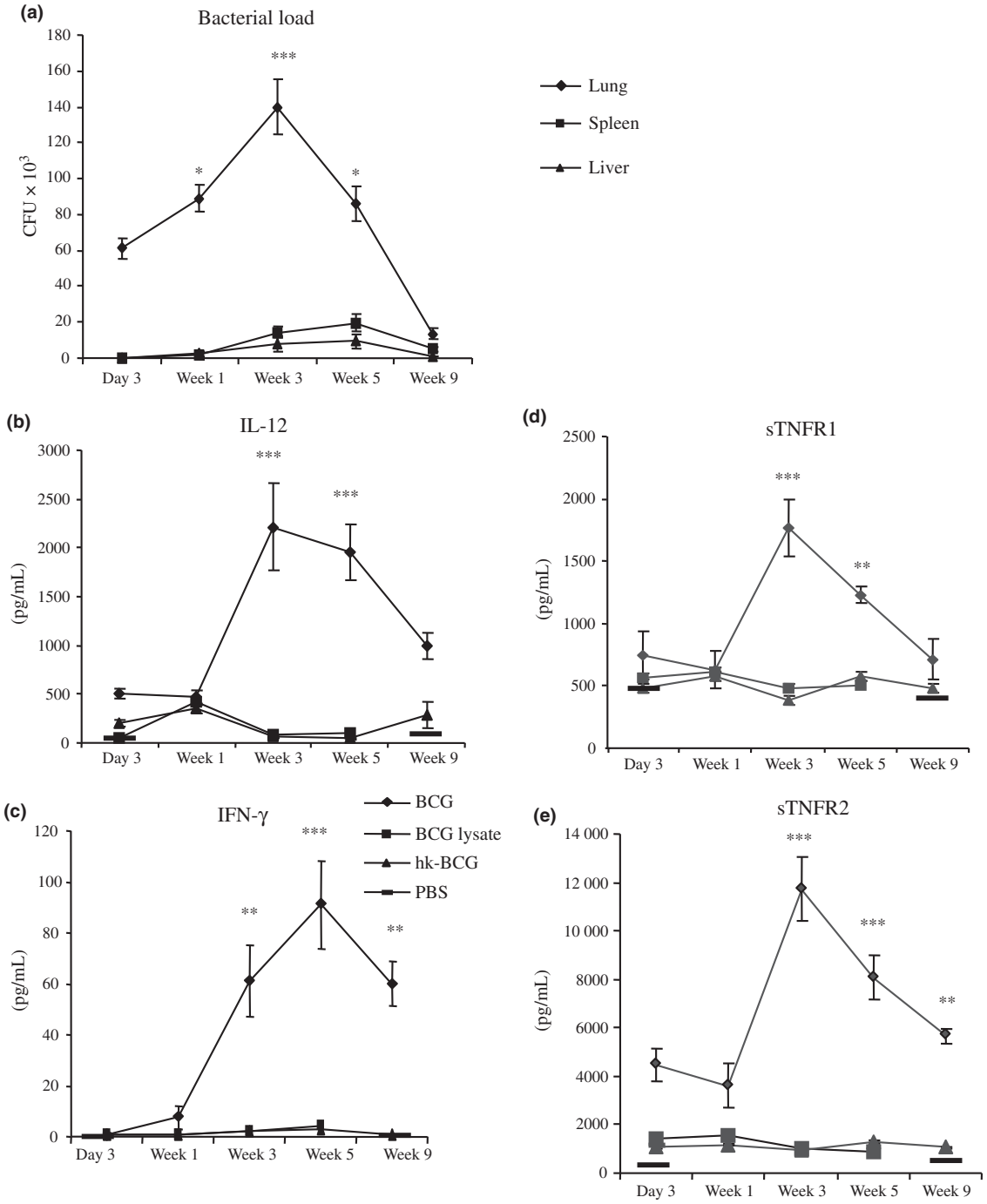

FIG. 1. Intranasal infection, but not treatment of mice with non-replicating BCG, induces elevated levels of IL-12 and IFN-γ, STNFRs in the lungs. Mice were infected intranasally with 10^7 CFU of BCG or treated with corresponding amounts of BCG lysate or heat-killed (BCG) (hk-BCG), and control mice were treated with PBS. Organs (lung, spleen, and liver) and BAL fluid were collected at day 3, weeks 1, 3, 5 and 9 after infection or treatment with hk-BCG, at day 3, weeks 1, 3 and 5 after treatment with BCG lysate, or at day 3 and week 9 for PBS-treated mice. Serial dilutions of lung, spleen and liver were plated on Middlebrook 7H11 agar, and bacterial loads were determined 2–3 weeks after plating (a). Levels of IL-12 (b), IFN-γ (c), sTNFR1 and sTNFR2 (d, e) in BAL fluid were measured with standard ELISA kits, using single sample dilutions of 1 : 5, 1 : 2, 1 : 5 and 1 : 50; mean concentrations are expressed as pg/mL. Results are expressed as mean CFU $\times$ 10^3 (a) and concentrations ± standard deviation (b–e) from four mice per group. The results shown are representative of three different experiments. *p <0.05, **p <0.01 and ***p <0.001 vs. D3.

detected in BAL fluid progressively increased during infection to week 3 ($p < 0.001$), and declined significantly by week 9. There was a positive relationship, but a weak correlation (r 0.48), between IL-12 levels in BAL fluid and bacterial growth in the lungs (Fig. 1a). The highest level of IFN-γ in BAL fluid (week 5), unlike that of IL-12 (week 3), did not exactly coincide with the peak bacterial growth in the lungs (week 3), but was indicative of active infection. In contrast to IL-12 or IFN-γ, minimal amounts of TNF were detected at all time-points (data not shown).

As TNF was barely detectable in BAL fluid, we reasoned that elevated levels of sTNFRs could result in TNF neutralization [21]. To assess this, we measured the levels of sTNFR1 and sTNFR2. As in the case of IL-12 and IFN-γ, live, but not non-replicating BCG induced increased levels of sTNFRs in the lungs, as detected in BAL fluid (Fig. 1d–e). There was a strong correlation between levels of sTNFR1 and sTNFR2 in BAL fluid (r 0.7 and r 0.68, respectively) and bacterial growth in the lungs. As compared with the lungs, there was significantly lower infectivity of the spleen or liver after intranasal infection of mice with BCG (Fig. 1a). Similar results were obtained in animals infected intravenously, even though bacterial growth in the spleen and liver increased (data not shown).

Elevated levels of IL-12 and sTNFRs in serum are more indicative of exposure to mycobacterial antigens than active infection

Peripheral blood is used for assessing the state of infection in several diseases. To determine whether serum levels of IL-12 or sTNFRs could be used in the same way as BAL fluid levels to predict an ongoing mycobacterial infection, sera collected from the tail vein at the time-points indicated above were analysed for the presence of cytokines. Increased levels of IL-12 and sTNFRs were found in serum, not only after infection, but also after treatment of mice with non-replicating BCG (Fig. 2a–c). There was no direct relationship between IL-12 or sTNFR level and bacterial growth (Fig. 1a) in the lungs, spleen, or liver. In these experiments, TNF and IFN-γ were undetectable in serum. These results suggest that the detection of cytokines or soluble receptors in serum may not be reflective of bacterial growth in the lungs, and therefore may not be reliable for prediction of an ongoing mycobacterial infection.

Reactivation of mycobacterial infection results in elevated levels of sTNFRs in BAL fluid

During the course of their lives, 5–10% of people latently infected with *M. tuberculosis* experience reactivation of their infection as a consequence of immunosuppression, resulting in increased bacterial growth in the lungs and other organs. In animal experiments, reactivation of controlled mycobacterial infection has been achieved by the administration of DXM [20], a corticosteroid that suppresses the effector functions of T-cells, which are central to the control of infection. To determine whether increased bacterial growth in the lungs would result in elevated levels of sTNFRs in BAL fluid, previously infected mice, that have controlled their infection (week 10 after intranasal infection), were treated with DXM as described above, and BAL fluid and serum were analysed for the presence of sTNFRs.

Administration of DXM resulted in increased bacterial growth in the lungs (Fig. 3a) by week 2 after treatment. There was also a slight but non-significant increase in bacterial load in the spleen and liver (data not shown). Soluble TNFR levels increased significantly in BAL fluid 2 weeks after DXM treatment (Fig. 3b–c), and this correlated strongly with bacterial growth in the lungs (r 0.9 and r 0.7 for sTNFR1 and sTNFR2, respectively). In contrast, sTNFR levels in serum after DXM treatment were comparable with those in untreated mice (data not shown). These results confirm our observation that sTNFR levels in BAL fluid correlate with bacterial growth in the lungs, and higher sTNFR levels may be indicative of an ongoing mycobacterial infection.

Mycobacterium-specific antibodies can be detected in BAL fluid and serum, but association with active infection in the lungs is more clearly correlated with BAL fluid antibodies

Thus far, our results have demonstrated a correlation between mycobacterial growth in the lungs and sTNFR levels in the lung microenvironment. Although there was a good correlation between sTNFR levels and active infection, this parameter is generally a marker of cell activation, and is not specific for mycobacterial infection. To confirm that infection was caused by mycobacteria, we analysed BAL fluid and serum for the presence of specific IgG and IgA antibodies, using BCG lysate as antigen. Only infected mice produced detectable levels of IgG antibodies in BAL fluid and serum (Fig. 4a–b), and the levels increased over time. BCG-specific IgA was detected in BAL fluid, but not in serum, and had similar kinetics to IgG (Fig. 4a).

Next, we determined whether detection of antibodies to single mycobacterial antigens in BAL fluid and serum could be used to predict an ongoing infection. As was found for BCG lysate, only active infection resulted in production of antigen-specific IgG or IgA in BAL fluid (Fig. 4c–d). In the serum, IgG but not IgA to single antigens was detectable after infection with BCG (data not shown). However, moderate amounts of IgG were detectable in sera obtained from mice treated with non-replicating BCG (Fig. 4e), indicating

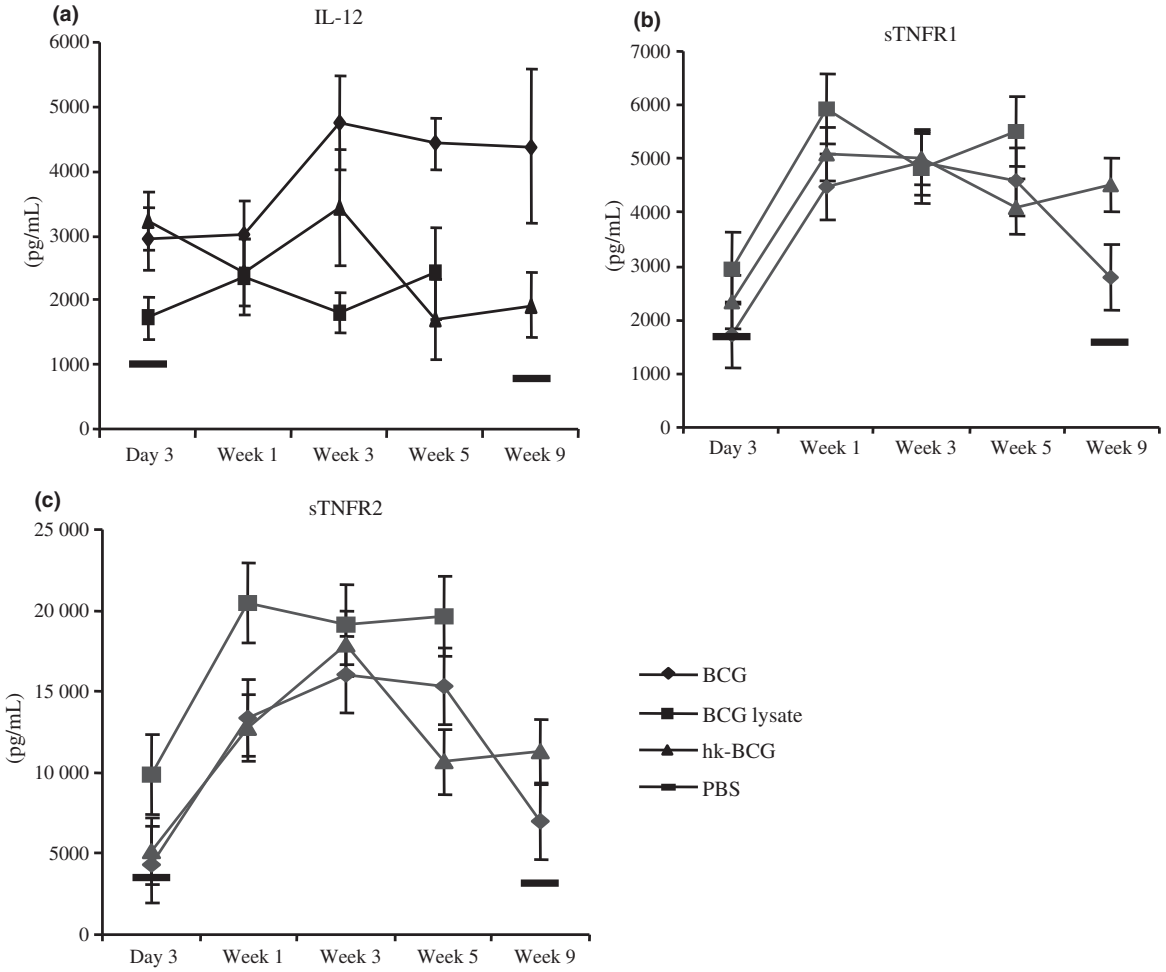

FIG. 2. Elevated levels of IL-12 and sTNFRs in serum are more indicative of exposure to mycobacterial antigens than of active infection. Sera from mice infected with live or non-replicating BCG as described in Fig. 1 were analysed for the presence of IL-12 (a), sTNFR1 (b) and sTNFR2 (c), with standard ELISA kits, using single sample dilutions of 1 : 10, 1 : 40 and 1 : 100; mean concentrations are expressed as pg/mL. Results are expressed as mean concentration ± standard deviation (a–c) from four mice per group. The results shown are representative of three different experiments. hk-BCG, heat-killed BCG; PBS, phosphate-buffered saline.

that serum levels may not be absolutely correlated with active infection. Overall, these data suggest that detection of antibodies to multiple or single mycobacterial antigens in BAL fluid, especially IgA, may be predictive of an ongoing infection in the lungs.

Discussion

The biological complexity of *M. tuberculosis* infection means that the use of a single immunological marker to predict of infection or disease activity would probably have limited diagnostic value, and analysis of several biomarkers would offer the possibility of improved diagnosis. In this study, we have assessed elements of the Th1 immune profile together with specific antibodies in the evaluation of the status of mycobacterial infection in mice. We have also demonstrated that, in TB, evaluation of local immune responses in the respiratory tract is more reliable than serum studies.

Over the years, the majority of studies that have investigated cytokines or their soluble receptors as markers of active disease in TB patients have looked mainly at the serum [13,14]. However, *M. tuberculosis* infection occurs more frequently via the respiratory tract, and currently there is a belief that in TB, as well as in other lung diseases, the markers of disease activity need to be measured locally in the lungs, and not in peripheral blood. In the present study, elevated levels of IL-12 or sTNFRs in serum were associated with exposure to mycobacterial antigens in general, and not exclusively to active infection. In contrast, elevated levels of

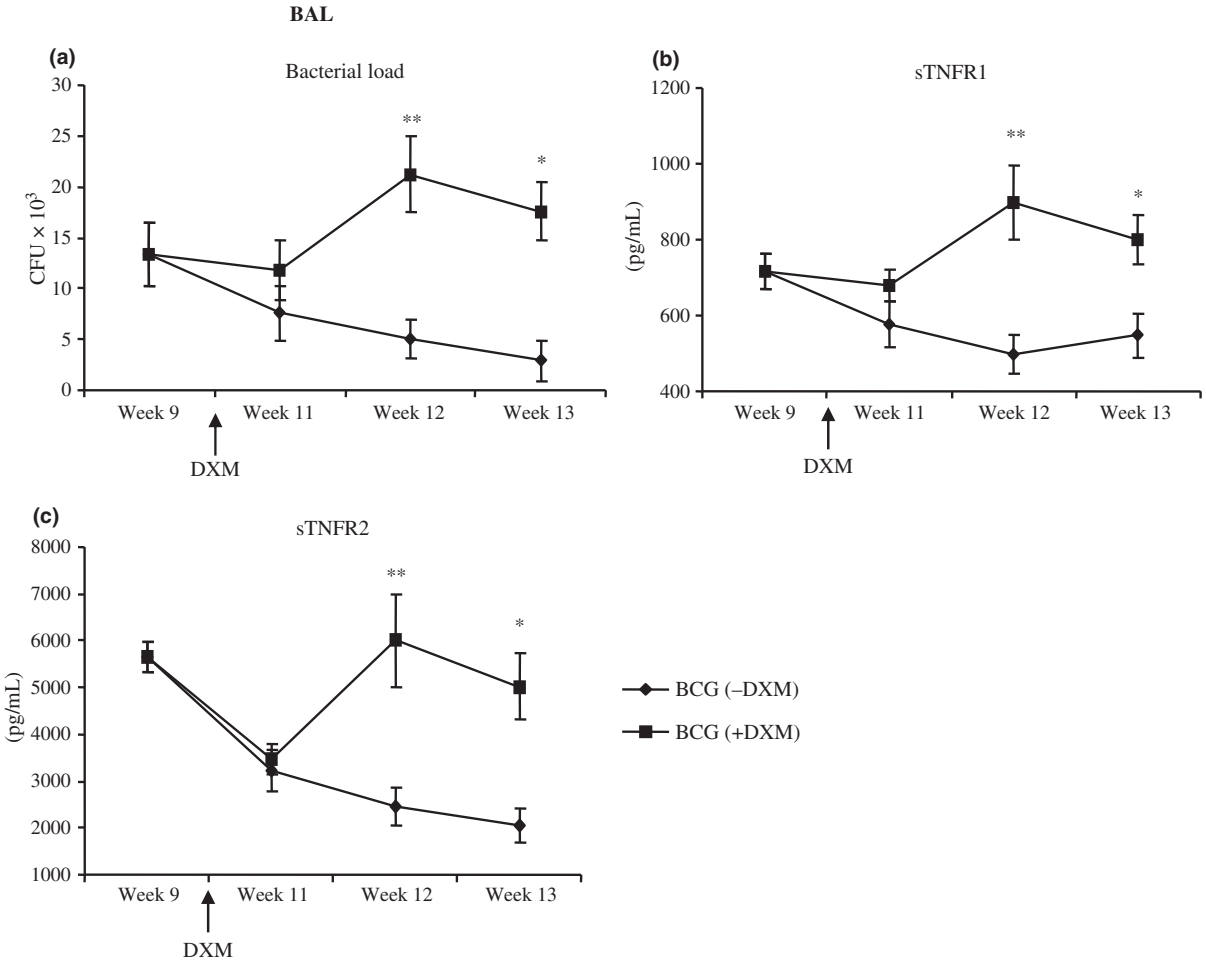

FIG. 3. Reactivation of mycobacterial infection results in elevated levels of sTNFRs in BAL fluid. Mice with controlled BCG growth in the lungs at week 10 after intranasal infection with 10^7 CFU of BCG were injected intraperitoneally with 6 mg of dexamethasone (DXM) per kilogram body weight every other day for a total of four injections. Organs (lung, spleen, and liver) and BAL fluid were collected at weeks 1–3 after DXM treatment. Serial dilutions of lungs were plated on Middlebrook 7H11 agar, and bacterial loads were determined 2–3 weeks after plating (a). BAL fluid was assayed as described above for detection of sTNFRs; mean concentrations expressed as pg/mL (b–c). Results are expressed as mean CFU $\times$ 10^3 (a) or concentration ± standard deviation (b–c) from four mice per group. The results shown are representative of three different experiments. *p <0.05 and **p <0.01 vs. w11.

IL-12 and IFN-γ, or the shedding of sTNFRs, in BAL fluid depended on the infection status. Also, in humans, several studies have directly demonstrated a relationship between cytokine production in the lungs and active TB, usually based on cytokine release by mononuclear cells at the site of infection [22,23]. However, in these studies, a TB patient was defined as any patient with a chest radiograph suggestive of TB, regardless of human immunodeficiency virus status. Thus, a possible contribution from some other co-infections could not be entirely excluded [24].

To confirm that the infective agent was BCG, we analysed mycobacterium-specific antibodies in BAL fluid and serum after active infection of mice, or treatment with non-replicating BCG lysate or single mycobacterial antigens were used for detection of antibodies. The higher levels of antibodies found in both BAL fluid and serum at week 9 after BCG infection, as compared with the early time-points, were expected. In contrast, non-replicating antigens would need continuous priming and/or help from relevant adjuvants to be optimally immunogenic. This may explain the inability of the non-replicating BCG administered as single doses to induce detectable antibodies in BAL fluid. Nevertheless, detectable levels of specific IgG were measured in serum of mice treated with non-replicating BCG. One explanation could be that the amounts of the single antigens in the coated plates were higher when they were used alone than when

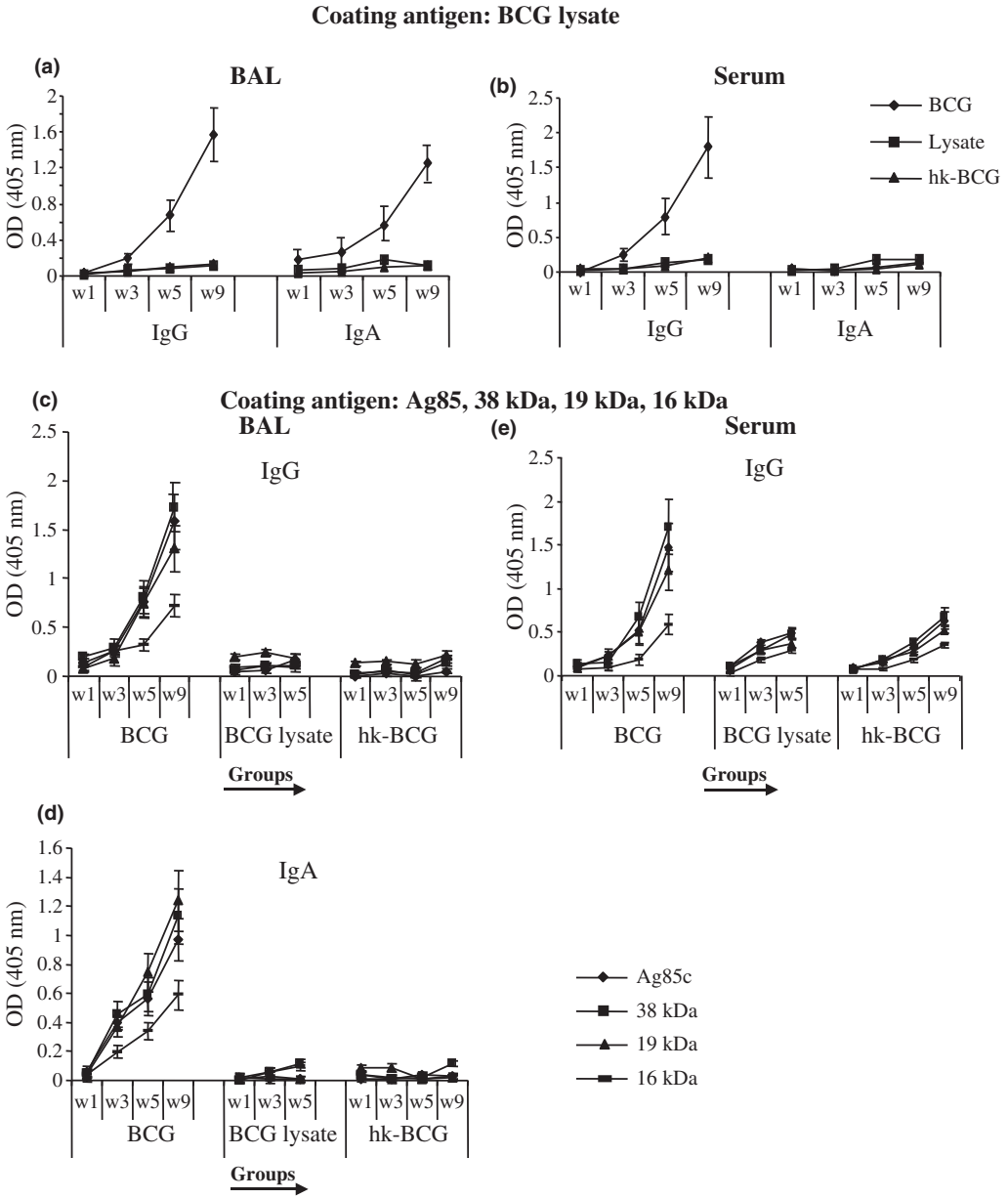

FIG. 4. Intranasal infection, but not treatment of mice with non-replicating BCG, results in production of BCG or antigen-specific antibodies in BAL fluid and serum. Mice were infected intranasally with 10^7 CFU of BCG or treated with 10^7 CFU of non-replicating BCG, and BAL fluid and sera were collected as above. (a) Microtitre plates were coated with 20 mg/L of BCG lysate as antigen ON in bicarbonate buffer (pH 9.6), and the amounts of BCG-specific antibodies were measured in BAL fluid and serum, using single sample dilutions of 1 : 25 and 1 : 50 for IgA and IgG in BAL fluid, and 1 : 2000 for IgG in serum. (b) Ag85, 38 kDa, 19 kDa or 16 kDa (2 mg/L) were used singly as coating antigens, as described for detection of antigen-specific antibodies in BAL fluid (c, d) and serum (e). Optical density (OD) was read at 405 nm, and the results are expressed as mean OD from four mice per group. The results shown are representative of three different experiments.

plates were coated with the whole lysate, when competition with other molecules for binding would obviously be greater.

In our study, IgA was detectable only in BAL fluid. IgA is the major immunoglobulin in mucosal secretions, and our group [6] and others [25] have demonstrated the induction of IgA in mucosal secretions after intranasal immunization with mycobacterial antigens, or infection with mycobacteria, respectively. The four antigens selected have been commonly used for the serodiagnosis of TB [12,16–18]. In contrast to

Ag85, 38 kDa or 19 kDa, lower amounts of anti-16 kDa antibodies were detectable in BAL fluid and serum. The 16 kDa antigen is a cytosolic regulatory protein specific to the *M. tuberculosis* complex and is expressed during latency [26]. A systematic analysis of humoral immune responses of TB patients has shown that the profiles of antigenic proteins of *M. tuberculosis* recognized by antibodies differ at different stages of infection and disease progression [27]. This suggests that an accurate diagnostic test for TB will need to be based on a combination of antigens.

It is clear that measurement of immune markers in BAL fluid may be technically demanding in resource-limited settings. Therefore, a methodology to perform similar studies in other mucosal secretions such as saliva would be rapid, simple, inexpensive and non-invasive in comparison with obtaining BAL fluid. This may be possible, because in previous studies we have demonstrated the presence of specific antibodies both in BAL fluid and in saliva after intranasal immunization with mycobacterial antigens [28]. Moreover, some human studies have revealed IgA antibodies specific for the 38 kDa mycobacterial antigen in the saliva of children aged 1–15 years [29]. For this reason, we are working to improve our detection assays in saliva. In conclusion, detection of a tailored combination of mycobacterium-specific antibodies and cellular markers in the respiratory tract will be useful for the immunodiagnosis of TB, and may help in the prediction of the clinical course of disease.

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Transparency Declaration

The study was supported by grants from the Swedish Institute and Hjärt-Lungfonden. The results reported in this article have been generated without any conflict of interest with any commercial or other enterprise.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Intravenous infection of mice with BCG results in elevated levels of sTNFR in BAL which correlate with bacterial growth in the lungs.

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